RETINAL VASCULATURE IN THE AGED*

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THE unique opportunity to examine the local vasculature in the living is available in the human eye through ophthalmoscopy. At present, clinical observation of the human fundus may be correlated with pathological findings by newer methods of tissue preparation. This presentation will help to demonstrate such clinicopathological relationships.

Previous studies[1] have shown that the macular area in the aged eye is vulnerable to degenerative changes, cyst formation, retinal atrophy and pigment cell migration, with no visible evidence of changes in the adjacent choroid. It was thought that these senile changes, and those associated with diabetes mellitus, hypertension, nephritis and arteriosclerosis, might be related to changes in the retinal circulation in and around the macula. The new technique for study of the retinal circulation described by Kuwabara and Cogan[2] seemed to be a method of value in this direction. Flat preparations of the retina, prepared and stained with periodic acid-Schiff's reagent as described by Friedenwald[3] and injected specimens of retinal vasculature as described by Ashton[4] had been tried but had not been sufficiently informative in our hands.

The material for this investigation was obtained at the Hospital and Home for Aged and Infirm Hebrews of New York. It consists of 115 eyes removed at postmortem from patients ranging in age from 70 to 96 years at time of death. The eyes were enucleated as soon after death as possible, usually within the first 12 hours, and frequently sooner. They were placed in 10 per cent neutral formalin solution until ready for sectioning. The length of time the eye remained in formalin did not seem to impair the tissue for study up to about two years.

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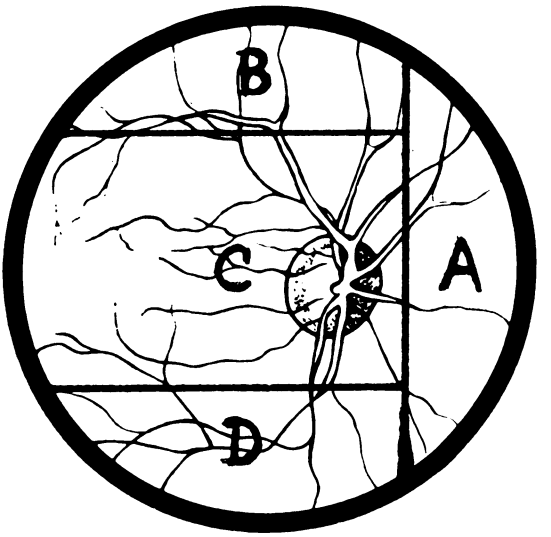
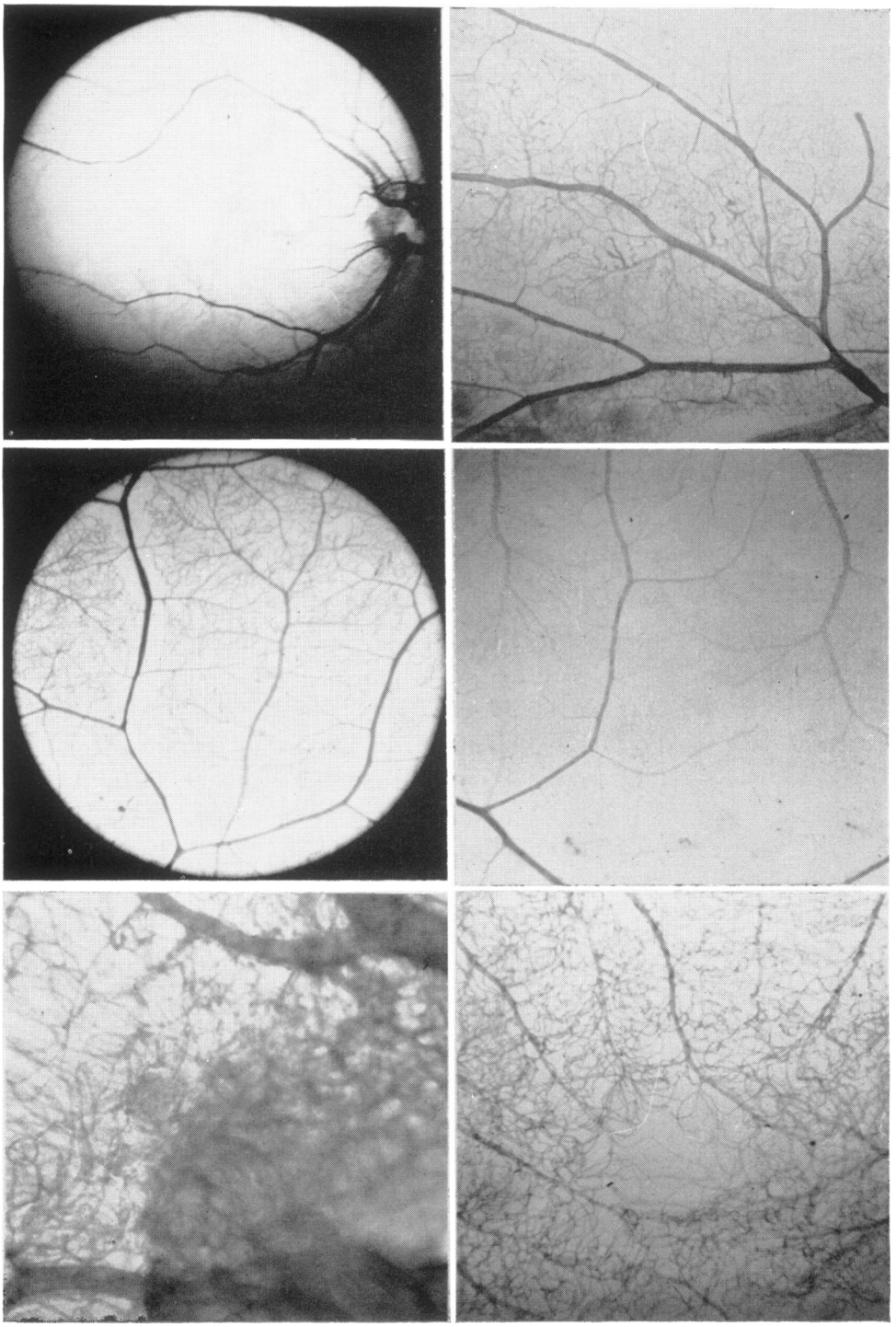


Fig. 1. Method of sectioning the posterior part of the eyeball.

Later than that, the retina did not always digest as easily nor take the stain as well as fresher tissue.

The anterior segment of the eye, containing the cornea, lens and ciliary body, was cut away at a level just in front of the ora serrata. The vitreous was washed out as much as possible with a thin stream of distilled water. The posterior half of the globe was cut in four sections with a sharp razor (Figure 1). The nasal portion (A) was cut close to the nerve. The upper and lower parts (B and D) of the temporal portion were then cut away leaving (C), the central portion of the retina containing the optic disc, the major vessels, the macular area and a portion of the periphery. The retina was peeled off the underlying choroid from each of these segments. Where the retina was adherent to the disc, it had to be separated with a sharp razor. These retinal fragments were then washed in distilled water overnight—with frequent changes of the water. They were then subjected to trypsin digestion using a solution of 3 per cent trypsin (Difco 1:250) and 0.15 M. tris buffer, pH 7.8 and incubated at 37°C for 1½ to 3 hours. The digestion is usually sufficient in this length of time and is indicated by the solution becoming cloudy and the tissue showing signs of disintegration. The tissue is removed with a bent iris repositor and put into distilled water where it is gently



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shaken to remove all digested tissue. While still in distilled water, and under a plastic cover to prevent dust accumulation, the internal limiting membrane is picked up with a fine forceps and carefully peeled off. This procedure requires considerable patience and dexterity and was often done under a dissecting microscope giving a magnification of 20x. This was done to minimize injury to the macular area where the retina is thinnest. The thin film of blood vessels was placed on a glass slide and allowed to dry. Generally, staining was done with periodic acid-Schiff's reagent, and counterstaining with hematoxylin. Several specimens were stained with Masson's trichrome stain to see if any further information could be obtained in this way.

Not all eyes were successfully processed, especially at the beginning, when several eyes had to be sacrificed for the sake of acquiring experience with the method. We are grateful to Dr. Kuwabara and Dr. Cogan for their advice and assistance in helping us to achieve better results and to perfect the technique.

We concentrated on the macular area because of our interest in macular degeneration, though all other areas were studied. Several interesting specimens were found associated with vascular disturbances. These included one eye with complete occlusion of the central retinal artery, and one eye with an occlusion of a branch of the retinal artery. There were also a number of eyes with changes due to diabetes, hypertension and glaucoma.

The clinical background of the patients had been noted during their stay at the Hospital and Home for Aged and Infirm Hebrews. This included annual medical check-ups and all necessary laboratory studies. Eye examinations were done on all new admissions and rechecked annually. If eye illnesses were found, treatment and observations were made as often as necessary in the eye clinic. Unfortunately, some patients had no eye examinations at the Home because they were too sick

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Fig. 2. (Top left) Low power view of retinal blood vessels and optic disc. Mag. ocular 8x, objective 1.5.

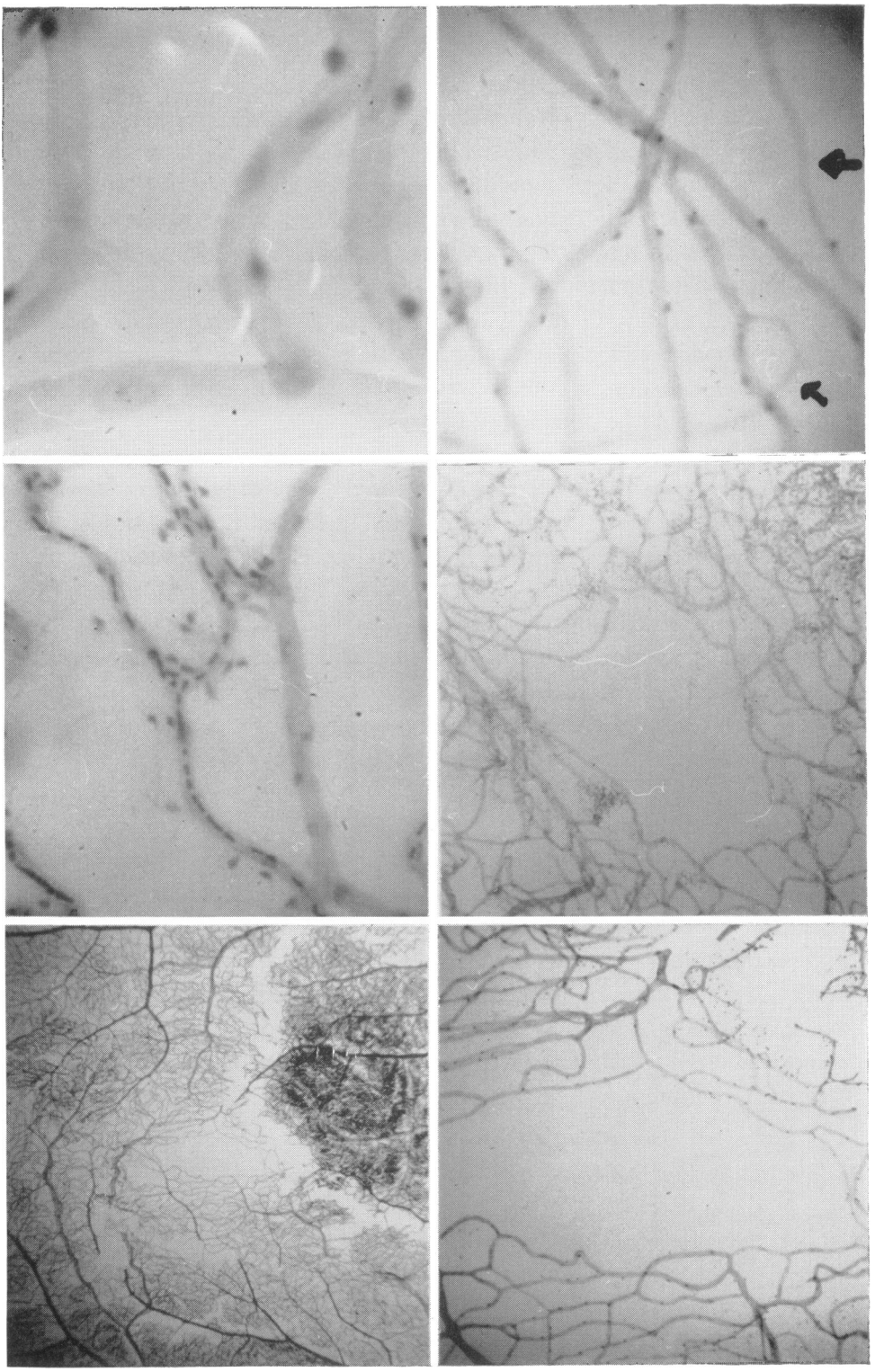
Fig. 3. (Top right) Dichotomous and side branching of the retinal arteries. Arteries stain darker. Mag. ocular 8x, objective 10x.

Fig. 4. (Middle left) Capillary-free zone around arteries, midperipheral section. Low Power. Mag. ocular 8x, objective 2.5x.

Fig. 5. (Middle right) Peripheral retina, showing fewer capillaries and continuity of circulation from arteriole to venule. Mag. ocular 8x, objective 10x.

Fig. 6. (Lower left) Optic disc and surrounding dense capillary network. Mag. Ocular 8x, objective 10x.

Fig. 7. (Lower right) Macular area. Note few capillaries traversing fovea. Mag. Ocular 8x, objective 2.5x.



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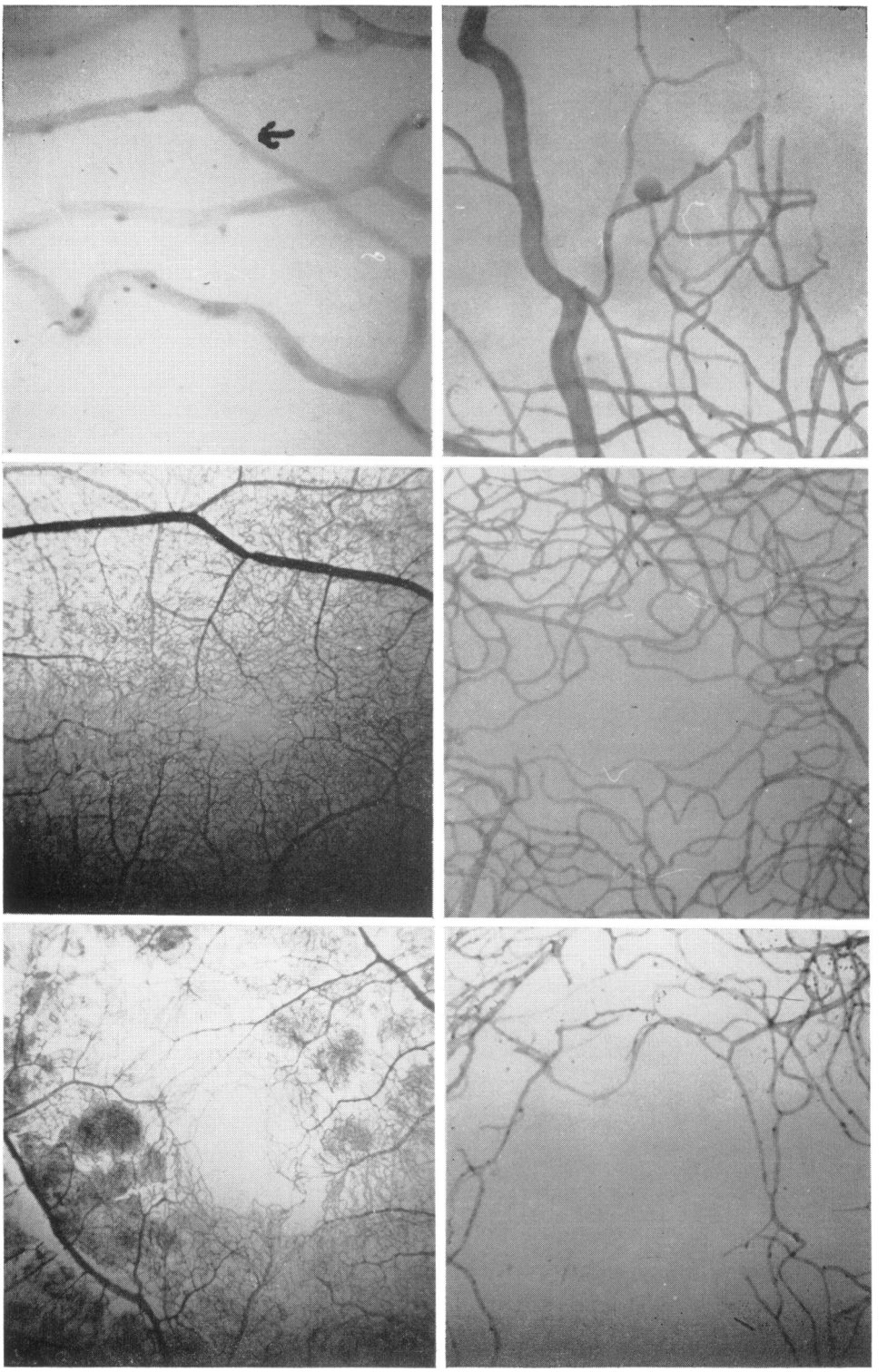
on admission, and they were there for too brief an interval before death supervened. Wherever possible, previous eye examinations before entering the Home were investigated to complete the clinical picture.

Of the 115 eyes processed, 59 good to fair specimens of the macular area were obtained. Nineteen of these had a clinical history of senile macular degeneration of varying degrees. This represents 32 per cent of this group of cases and is in agreement with the percentage of macular degeneration noted in our original statistical study of the eye in old age.[5] Of the remaining 56 eyes, 14 eyes had a history of senile macular degeneration, making a total of 33 eyes or 29 per cent of the total number of eyes examined. This figure may be low because a few patients had had no eye examination before death.

Description of Normal Retinal Vasculature

The appearance of the retinal vasculature by this method of processing has been amply described by Cogan, Kuwabara and their associates.[2, 6-9] Our findings are substantially in agreement with theirs. The over-all appearance of the retinal vasculature on the microscopic slide in low power is very similar to the ophthalmoscopic picture in the living eye (Figure 2). The retinal arteries stain more deeply purplish red and can thus be readily distinguished from the veins. The branching of the arteries is of two types: dichotomous branching of about equal calibre as the vessels advance toward the periphery; and side branching coming off directly from the main vessels, usually of much smaller calibre and usually at right angles to the direction of the main vessel (Figure 3). Occasionally, the direction of these smaller vessels is angulated slightly forward and may even be slightly backward from the direction of the main artery. Around the artery there is a zone free of capillaries. This capillary-free zone is more easily seen as the artery advances toward the periphery (Figure 4). The terminal branches of the arterioles break up eventually at the ora serrata into capillary loops which anastomose directly with capillaries from the venous side of the circulation. The

Fig. 8. (Top left) Capillaries under oil immersion showing nuclei of endothelial and mural cells. Mag. Ocular 8x, objective 100x.
Fig. 9. (Top right) Capillary net showing intercapillary strands. Mag. Ocular 8x, objective 40x.
Fig. 10. (Middle left) Capillaries with red blood cells. Mag. Ocular 8x, objective 40x.
Fig. 11. (Middle right) Normal macular area. Mag. Ocular 8x, objective 10x.
Fig. 12. (Lower left) Mamular area in early macular degeneration. Mag. Ocular 8x, objective 2.5x.
Fig. 13. (Lower right) Foveal area. Mag. Ocular 8x, objective 10x.



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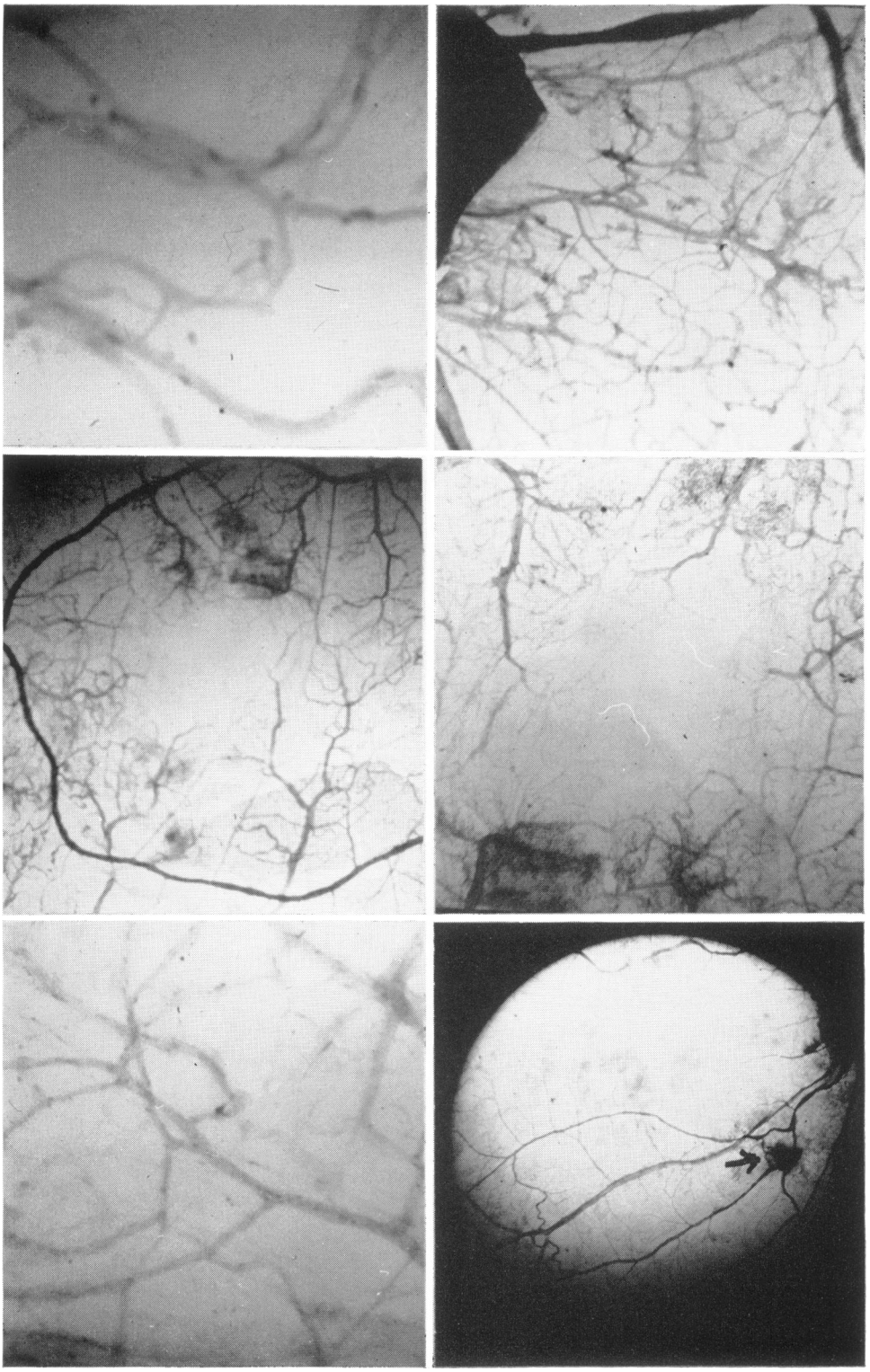
continuity of the artery to arteriole to capillary and then from the prevenule capillary to vein can be readily followed, especially in the periphery where the capillaries are much fewer in number and not so closely meshed as in other parts of the retina (Figure 5). The major arteries and veins are in close proximity as they advance from the disc to the periphery. Throughout the retina at the level of arteriole and venule there is a definite pattern of alternation, so that arterial capillaries drain directly into local venous capillaries.

The capillary bed represents the most interesting part of the retinal vasculature by this method of study and our discussion from this point of view will deal mainly with the capillaries. The area of greatest capillary density is around the disc (Figure 6). This was pointed out by Michaelson,[10] whose book *The Retinal Circulation in Man and Animals* is basic reading for all who are interested in this subject. He describes four layers of vessels in the peripapillary area. The capillary density diminshes gradually as we advance to mid-periphery and periphery, where a single layer of widely separated capillaries can be seen. In the macular area the capillaries are fairly dense but thin down perceptibly as the fovea is approached. The foveal area is essentially free of capillaries and measures between 0.4 and 0.6 mm. in diameter. Foveal spots were seen in several eyes in which capillaries cross the fovea, so that this area may not be entirely free of capillaries (Figure 7). The capillaries vary in calibre from 20 to 150 microns, the average being 45 to 50 microns in width. They stain a pale violaceous pink with the PAS stain. The demonstrable nuclei are of two types (Figure 8). The endothelial cell nucleus is stained a pale bluish purple and it is elongated oval in shape with the long axis parallel to the direction of the capillary. The other nucleus is that of the mural cell which is really within the basement membrane of the capillary. This nucleus is small, round or oval-shaped and takes a dark purplish stain. It appears to protrude away from

Fig. 14. (Top left) Capillary area with intercapillary strand. Mag. Ocular 8x, objective 40x.
Fig. 15. (Top right) Capillary showing aneurysmal dilatation. Mag. Ocular 8x, objective 20x.
Fig. 16. (Middle left) Macular area in early macular degeneration. Mag. Ocular 8x, objective 2.5x.
Fig. 17. (Middle right) Foveal area showing numerous intercapillary strands. Mag. Ocular 8x, objective 10x.
Fig. 18. (Lower left) Macular degeneration in advanced case. Mag. Ocular 8x, objective 2.5x.
Fig. 19. (Lower right) Foveal area showing considerable enlargement, acellularity and numerous intercapillary strands. Mag. Ocular 8x, objective 10x.



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the capillary wall, but electron microscopy has shown that it is really surrounded by part of the basement membrane, which has split into two parts to enclose it.[11] In addition to the capillaries, intercapillary strands are seen of varying thickness, but all much thinner than the capillaries (Figure 9). Occasionally, these strands have nuclei associated with them, usually at the ends of the strands, but they may also be present at any point along their extent. These intercapillary strands have given rise to much controversy. The thicker ones, especially those with nuclei, have been thought to be obliterated capillaries. The very thin ones without nuclei have been interpreted as strands of glial tissue, or mesodermal tissue, or congenital strands that failed to develop into capillaries. Our interpretation from the study of our specimens is that they are obliterated capillaries secondary to failure of blood flow. Figure 10 demonstrates the presence of red blood cells inside the capillaries. This is a good indication of patency of the capillaries, but cannot always be seen.

The normal macula and foveal area are demonstrated in Figure 11. The fovea measures 0.5 mm. in diameter and is surrounded by a fairly dense plexus of capillaries. The capillaries are mainly uniform in calibre and there are few if any intercapillary strands. The two types of nuclei are present in the capillaries which anastomose freely with one another. Kuwabara and others have found certain characteristics in the eyes of the aged. They are: 1) acellularity or loss of nuclei, chiefly endothelial nuclei (this may be present in a single capillary or in a cluster of capillaries); 2) diminution of staining capacity or achromophilia; 3) irregularity of contour of the capillaries. Our studies appear to confirm these findings, though not with a sense of certainty. It was difficult for us at times to be sure whether the accellularity and achromophilia were pathological findings or rather variables of the technique.

Fig. 20. (Top left) Capillaries at foveal margin showing intercapillary strands, acellularity and some achromophilia. Mag. Ocular 8x, objective 40x.

Fig. 21. (Top left) Total occlusion of central retinal artery. Note disorganization of capillary bed, accellularity, achromophilia, tortuosity and conversion into strand-like forms. Mag. Ocular 8x, objective 10x.

Fig. 22. (Middle left) Macular area in total occlusion of central retinal artery. Note enlargement of foveal area in addition to other capillary changes. Mag. Ocular 8x, objective 2.5x.

Fig. 23. (Middle right) Foveal area under higher power showing capillary disorganization. Mag. Ocular 8x, objective 5x.

Fig. 24. (Lower left) Capillary meshwork in midperiphery showing almost complete obliteration of all capillaries. Mag. Ocular 8x, objective 40x.

Fig. 25. (Lower right) Retinal vasculature showing area of occlusion of a branch of the central retinal artery. Mag. Ocular 8x, objective 1.5x.

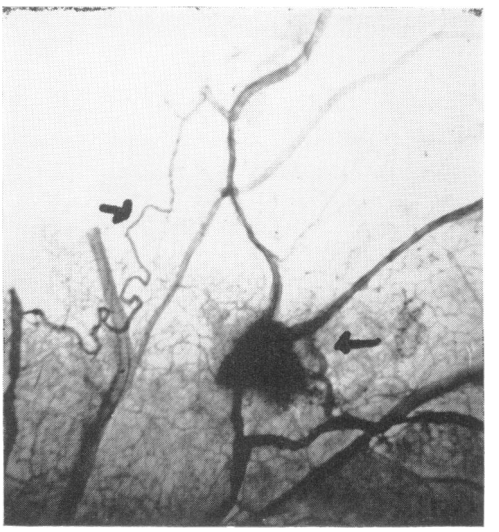


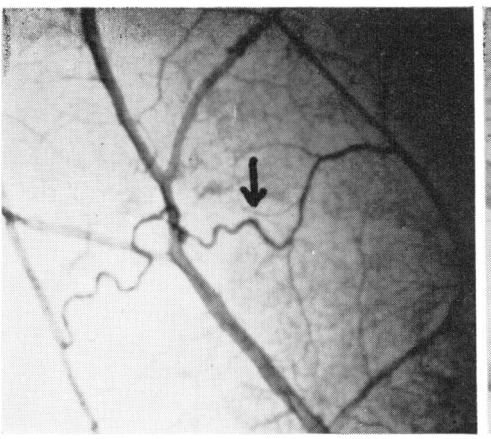
Fig. 26. Close-up of occluded artery. Two collateral vessels can be seen, one to the right and the other to the left of the occluded artery. Mag. Ocular 8x, objective 2.5x.

Senile Macular Degeneration

The following eyes will demonstrate the findings in patients with a clinical history of senile macular degeneration. The first eye (#910) is that of a female, age 87 at the time of death. The last vision recorded was 15/70 in this eye. She also had diabetes mellitus. The appearance is not much different from the normal aged eye. Several views under different magnification can be seen in Figures 12 to 15. They demonstrate the appearance of the fovea, the surrounding capillaries, the intercapillary strands and a few aneurysmal dilatations.

The second eye (#916) is from a female patient, age 79 at the time of death. The vision was 15/100 at the last clinical examination. It is similar to the first eye, and shows intercapillary strands very well (Figures 16 and 17).

In the above two cases, the appearance of the macular area in early or moderately advanced senile macular degeneration is not much different from the relatively normal aged eye. However, in the next eye (#908), that of a woman aged 91, whose vision was reduced to finger counting, the macular area shows much greater damage. Here there is widening of the foveal area, fewer capillaries around the fovea, loss of



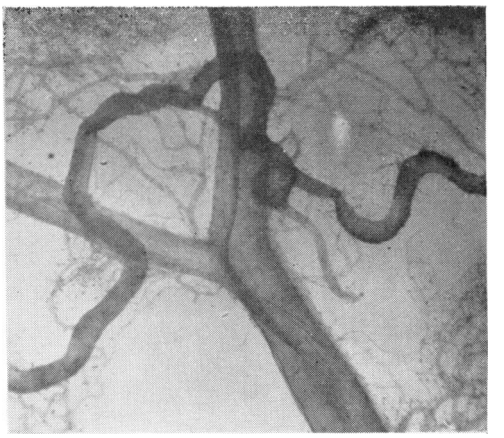


Fig. 27. (Left) A third collateral vessel joining two arterial branches distal to the occluded retinal artery. Mag. Ocular 8x, objective 10x.
Fig. 28. (Right) Close-up of collateral vessel, showing tortuosity, increased thickness, and possible communication with vein it crosses. Mag. Ocular 8x, objective 40x.

cellular elements in these capillaries and poor staining capacity (Figures 18 to 20).

From our observations it is difficult to say with certainty, except in advanced cases, that macular degeneration as seen clinically is due to changes in retinal vasculature, specifically, pathology in the capillary bed. We realize that functional changes cannot be demonstrated frequently in pathological specimens. The closure or even narrowing of a few capillaries in an area such as the macula may have a marked clinical effect. The oxygen requirement of the macular area is very great, and a diminution of this supply, even in small measure, may be locally detrimental. Further study of this area using definite criteria, comparing normal and abnormal maculae, will be done and reported at a later date.

Arterial Occlusion

Several eyes that we came across in our study were of special interest. They tend to confirm our belief that the closed or obliterated capillary is a distinct pathological entity. The first of these eyes is from a male patient (#911), aged 85 (Figures 21 to 24). The capillary plexus is reduced almost completely to strands of tissue having no nuclei and staining very poorly. All that can be well seen are the major arteries and veins at their main branches. This condition is widespread throughout

the retina. A severe loss of function of the retina was postulated from the appearance of the pathological specimen, and reference back to the clinical history confirmed the pathological diagnosis of occlusion of the central retinal artery. There was a clinical history of central retinal artery occlusion with reduction of vision to faintest light perception.

The next eye (#901) of even greater interest was from a female aged 86 with a history of an occlusion of a branch of the retinal artery (Figures 25 to 28). Here it can be seen that the capillary bed close to the point of occlusion shows completely obliterated capillaries. The capillaries in other areas are relatively normal. In addition, this eye also shows those areas where collateral circulation has been established to the region formerly supplied by the occluded vessel. It is our belief that this is the first time that such collateral circulation has been demonstrated pathologically. One cannot be absolutely sure that these collateral vessels, of which three can be seen clearly, are direct shunts from arteriole to arteriole. A more likely route is from arteriole to venule and then retrograde from venule to arteriole. The vessel becomes thicker and more dilated and takes a tortuous course. The tortuosity may be due to the development of this vessel from pre-existing capillaries. The thickening of the vessel wall and increased lumen would be expected with increased blood flow. The clinical history stated that the condition of the eye improved both functionally and ophthalmoscopically. At the time of diagnosis of superior temporal branch occlusion, exudates and edema were seen in a partially circinate appearance and vision was reduced to 15/50. Two years later, all the edema and exudates had disappeared and vision had improved to 15/25.

Summary and Conclusions

The retinal vasculature of 115 eyes of patients aged 70 to 96 years was examined by the trypsin digestion technique of Kuwabara and Cogan. Of this group, 59 good specimens were obtained of the macular area. Nineteen of these eyes had a clinical history of senile macular degeneration. The changes in the capillary plexus of vessels associated with aging and with senile macular degeneration were evaluated. In early cases of macular degeneration, hardly any changes were noted to distinguish them from the relatively normal aged macula. In late or advanced cases, certain changes were noted. These were an increase in the diameter of the foveal area, a diminution in the number of capillaries

around the fovea, increased acellularity and increased achromophilia. Further study of this area using more exact criteria is in progress.

Two cases, one of total occlusion of the central retinal artery, and one of an occlusion of a branch of the central retinal artery, were demonstrated. These cases led to the belief that the obliterated capillary is a definite pathological entity. Also, collateral arterial circulation in the retina was shown in three areas in the eye with branch occlusion.

Acknowledgment

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